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Review Article

Redefining interferon: the interferon-like antiviral effects of certain cytokines (interleukin-1, interferon-β_2, interferon-γ) may be indirect or side effects

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The name of the interferons

'What's in a name? That which
we call a rose
By any other name would smell
as sweet'

Interferon; Interleukin-1; Interleukin-6; B-cell stimulating factor-2 (BSF-2); Macrophage activating factor

Introduction

The name 'interferon' was given in 1957 by Isaacs and Lindenmann (1957) to a 'factor' responsible for interference between viruses. Interference itself had already been defined much earlier by Findlay and McCallum (1937) as the phenomenon whereby cells or intact animals infected with one virus are partially or completely resistant against superinfection by a second related or unrelated virus. It took several years of hard preparative and analytical work before proof became available that a single protein, and not a complex of interacting biochemical entities, was endowed with this new biological action. At that point the term interferon inevitably came into use to designate the molecule, rather than an ill-defined and possibly complex factor. As a next step it became clear that, in the course of

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virus infections, not a single but several species of molecules, each endowed with interferon activity were produced. It then became necessary to provide the term interferon with an optional plural 's' as well as to distinguish 'types' and 'subtypes' of interferon. Also, not only viruses but several other agents or substances were found to be able to induce production of these molecular species.

Meanwhile, since various broadly active antiviral factors were described in biological fluids of all sorts, it had become necessary to establish criteria for such factors to qualify as interferons. In 1966 (Lockart, 1966) 4 criteria were suggested for use in classifying an antiviral substance as an interferon. In 1973 (Lockart, 1973) these criteria were expanded to seven, as follows: (1) The virus inhibitor must be a protein; (2) its antiviral effect must not result from non-specific toxic effects on cells; (3) it must be active against a wide range of unrelated viruses; (4) it must inhibit virus replication through an intracellular effect which must involve synthesis by the cells of both RNA and protein. An inhibitor which meets all these criteria can be classed with certainty as an interferon. In addition, most interferons have the following properties; (5) they are found in the body or in tissue cultures only when some stimulating substance has been added (there are however a few reports of spontaneous interferon production – autogenous interferon); (6) they are usually much more active in cells of the homologous species or phylogenetic order than in other cells; (7) they are usually stable at pH 2.

In 1980 (Stewart et al., 1980) these critera were condensed into a brief definition: '...To qualify as an interferon a factor must be a protein which exerts virus nonspecific, antiviral activity at least in homologous cells through cellular metabolic processes involving synthesis of both RNA and proteins...'. In 1984, when the amino-acid sequences of several interferon proteins had become known, Knight (1984) commented as follows: 'It is ironic that on the molecular level the definition of an interferon has become less rather than more specific. No homologous amino-acid sequence nor obvious similar secondary or tertiary structures have been found to unify the human interferons, thus suggesting that the definition of an interferon will remain a biological and not a chemical definition.'

The establishment and repeated confirmation of these criteria has had the merit to preclude a number of dubious antiviral factors from being called interferons. However, since the criteria constituted only necessary, but not sufficient requirements, they probably were unsuitable to exclude all molecules that are not interferons.

Here I will discuss 3 examples of antivirally active molecules to which these criteria apply, but which are not or may not be true interferons. These molecules are IL-1, BSF-2 and IFN-γ.

Interleukin-1

Interleukin-1 is a cytokine produced mainly by stimulated monocytes (Dinarello, 1984). There are two types (IL-1α and -β) which share most, if not all, biological properties. These properties are multiple. All but very few of them were

discovered before it was recognized that they belonged to the same molecular entity. The interferon-like antiviral effect was no exception: it was discovered as an antiviral factor (22K factor) present in mitogen-stimulated human leukocyte cultures (Van Damme et al., 1981, 1983, 1984, 1985, 1987b). Such cultures were known to contain interferon-γ. However, careful analysis revealed that a second antiviral factor was present. Since its activity was blocked by antisera against IFN-β, it was originally believed that this factor was IFN-β or any IFN-β-related molecule (Van Damme et al., 1981, 1983). However, purification and molecular characterization showed that the factor corresponded to a protein with a sequence quite different from that of IFN-β but identical to that of IL-1β (Van Damme et al., 1984). It was thereby demonstrated that IL-1β possesses antiviral activity. In fact the molecule IL-1β meets the four classical criteria for being called an interferon. However, not nomenclature but mechanism of action has been the main concern triggered by this finding of IL-1 being antivirally active. The most intriguing element in the observations is the fact that the antiviral effect of IL-1 can be neutralized by antibodies directed against IFN-β. The reverse is not true. Also, IL-1 does not bind to immobilized antibodies against IFN-β and there are no homology regions in the amino-acid sequences of IFN-β and IL-1.

In principle, there are 4 possible explanations for this strange state of affairs.

IL-1 is a bona fide interferon. Although it does not share amino-acid sequence homology with IFN-β, there may be conformational epitopes which explain one-directional neutralization without binding. Although this type of serological relationship seems unlikely, it should be mentioned that it has been invoked as an explanation for a similar state of affairs in the case of BSF-2 (see below).

IL-1 induces, directly or indirectly, the production of IFN-β or a serologically related antiviral factor which then acts back on cells to render them resistant against virus infection.

It is known that several, if not all biological effects of IL-1 are mediated by induction of intermediary biologically active factors (Dinarello et al., 1984). For one thing, IL-1 is considered as the physiological inducer of IL-2 (T-cell growth factor). IL-1 also is able to stimulate bone marrow adherent or stromal cells to produce colony stimulating factor(s) (Fibbe et al., 1986; Zucali et al., 1986). The B-cell stimulating effect of IL-1 seems to be due to induction of one or more B-cell growth and/or differentiation factors, one of which is BSF-2 (Content et al., 1985; Van Damme et al., 1987a, 1987c). Other active substances whose production is induced by IL-1 are: collagenase, prostaglandin synthetase, tissue-type plasminogen activator and urokinase. In view of this long list of factors which are inducible by IL-1, it would not be surprising to find that IL-1 would also stimulate synthesis of IFN-β. Experiments done to demonstrate such an effect have met with several difficulties (Van Damme et al., 1985). One is the fact that induction, if present, is very weak, both at the level of secreted protein and at the level of IFN-β-specific mRNA. A second difficulty is the fact that IL-1 is a potent inducer of BSF-2 (Content et al., 1985; Van Damme et al., 1987a, 1987c). This B-cell growth and differentiation factor happens to possess antiviral activity, which is the reason why it is also known under the name of IFN-β_2. Although this antiviral activity is quali-

tatively (i.e. serologically) indistinguishable from that of classical IFN-β (henceforth IFN-β_1), it is quantitatively much less pronounced as its specific activity is > 10 000-fold lower. Therefore, if one wants to explain the antiviral effect of IL-1 by its ability to induce IFN-β-like molecules one has the frustrating choice between weak induction of a strongly active IFN-β_1 or strong induction of a weakly active IFN-β_2 (= BSF-2).

IL-1 may act antivirally by inducing increased numbers of receptors for IFN-β. If IL-1 were able to cause an increase in the number or availability of receptors for IFN-β, and if small amounts of IFN-β, which would otherwise be insufficient to affect cells, were already present in cellular microenvironment, IL-1 would make the cells receptive to that constitutive interferon and thereby induce an antiviral state. There is evidence that certain cells, especially of fibroblastoid origin, may produce small amounts of IFN-β without really being stimulated to do so (Van Damme et al., 1985). Whether IL-1 does indeed cause increased numbers of IFN-β receptors has not been studied so far.

A last consideration is that the various hypotheses already formulated are not mutually exclusive. One could postulate, indeed, that IL-1 has some direct antiviral activity of its own but also synergizes with IFN-β_1 (present in the system and/or induced by IL-1) and with IFN-β_2 (induced by IL-1). Although some of the elements in this hypothesis are amenable to testing, evidence has so far not been brought forward.

In conclusion, the antiviral effect of IL-1 on cells is largely indirect involving the participation of IFN-β_1 and/or IFN-β_2 (BSF-2). We have no reason therefore to call IL-1 an interferon. The case of IL-1 should be a reason to introduce an additional criterion for a molecule to qualify as an interferon, namely requiring that the molecule should exert its antiviral action directly and not by inducing known interferon(s).

B-cell stimulating factor-2 (BSF-2 = 26K protein = IFN-β_2 IL-6)

Human BSF-2 is a factor primarily defined as being produced by T cells and endowed with the ability to promote Ig-synthesis by EBV-transformed human B-cells (Hirano et al., 1985, 1986). Recently, the protein corresponding to this factor has been obtained, characterized and found to be identical to a protein called 26K (Billiau, 1986; Sehgal et al., 1987). This protein has been known for several years to be produced by fibroblasts and claimed to be endowed with antiviral activity, which is the reason why it is referred to by some authors as IFN-β_2 (Weissenbach et al., 1979). The fibroblast-derived protein also possesses growth promoting activity for B-hybridoma and plasmacytoma cells (HPGF = hybridoma-plasmacytoma growth factor) (Van Damme et al., 1987c).

Having 4 names for the same molecular entity is an undesirable source of confusion and therefore it will be necessary in the future to discard at least 3 of the 4 current terms for the molecule. It has already been proposed that the molecule be renamed as interleukin-6 (Poupart et al., 1987). Here we will preferentially use

TABLE 1

Comparative biological effect and specific activities of the human 26 kDa protein.

Source of 26 kDa protein	Biological assay	Biologial units per mg protein	Comment and references
Normal fibroblasts, induced with IL-1	Growth promoting activity rat and mouse B-cell hybridoma cells	10^9	Preparation containing levels of protein detectable only by silver stain on slab gels (< 10μg/ml) titered 10 units/ml in growth promoting assay on B-cell hybridomas (Van Damme et al., 1987c)
	mouse B-cell plasmacytoma cells	10^7	Plasmacytoma cells are 100-fold less sensitive than hybridoma cells (Van Damme et al., 1987c)
	Interferon-like antiviral activity on human cells	$< 10^4$	Antiviral activity was never found (< 3 units/ml) even in preparations containing 80 000 units/ml of HGF activity (Van Damme et al., 1987b).
Leukemia virus-transformed human T-cell line, uninduced	Stimulation of Ig-secretion by EBV-transformed human B-cell lines	10^7	Hirano et al. (1985); 1 unit = 3 pmol
	Antiviral activity	Extremely low or nonexistent	Hirano et al. (1986 and personal communication)
Cloned cDNA, expressed and secreted by oocytes	Growth promoting activity rat and mouse B-cell hybridoma cells	2×10^8	Poupart et al. (1987); calculated on the basis of maximal protein synthesizing capacity of oocytes and on biological activity of secretion product
	Differentiation of CESS cells	2×10^7	
	Interferon-like activity on human cells	$< 10^2$	Poupart et al. (1987); no antiviral activity detectable in preparation containing 10^5 units/ml of HGF activity

the designation 26K protein since it is the most 'neutral' of designations. We will reflect on the reasons why (or why not) this molecule should be ranked among the interferons.

The claim for antiviral activity of the molecule is intimately linked with its first discovery as a substance produced by human fibroblasts (Content et al., 1982; Sehgal and Sagar, 1980; Weissenbach et al., 1979, 1980). Investigators involved in the cloning of classical IFN-β, found that stimulated human fibroblasts produced two populations of mRNA whose gene products in in vitro translation systems were precipitable with antisera raised against more-or-less pure IFN-β. One of these mRNAs was unambiguously found to correspond to classical IFN-β (IFN-β_1) by several criteria of which the following are only a few: (1) the amino-acid sequence, as derived from the cDNA sequence, matches that determined on the natural IFN-β_1 protein; (2) the in vitro translation gene product is precipitated by the purest available antisera against IFN-β_1; (3) the corresponding gene, like the genes of IFN-α has no introns and is localized on the same chromosome, both in man and in mouse. The second of the mRNA populations which appears in stimulated fibroblasts proved to code for an immunoprecipitable protein with quite different properties, and subject to different regulatory influences (Poupart et al., 1984).

In certain assay systems the in vitro translation product possessed interferon-like antiviral activity (Sehgal and Sagar 1980; Weissenbach et al., 1979, 1980). This activity was weak in that demonstrating it required the use of the most sensitive of bio-assays available. That the molecule does indeed possess antiviral activity was later confirmed in experiments using the gene product obtained by expression in heterologous cells (Revel et al., 1986; Zilberstein et al., 1986). The fact that demonstration of the antiviral activity requires either extremely sensitive bio-assays or concentrations of the product which have so far only been attainable by the use of recombinant DNA-derived materials, raises the inevitable question whether this antiviral activity is not rather a side-effect. Given the fact that the protein possesses at least two other biological activities, i.e. B-cell growth and B-cell differentiation promoting activity (Billiau, 1986, 1987; Hirano et al., 1982, 1985; Sehgal et al., 1987; Van Damme et al., 1987c), a comparison of specific activities (i.e. biological units per unit weight of product) is imperative. Presently available data are summarized in Table 1. Although the natural product was obtained in high concentrations (1.7×10^7 units of HGF activity per ml, or $> 1.7 \times 10^9$ units/mg protein) and in sufficiently pure form to reveal the N-terminal amino acid sequence, it did not possess antiviral activity ($< 10^2$ units/ml) in an assay system where classical IFN-β_1 had the expected specific antiviral activity of 10^8 units/ml. Accordingly, the specific antiviral activity of BSF-2 was estimated to be less than 10^4 units/mg protein. A similar discrepancy between high B-cell stimulatory effect and lack of antiviral effect was also seen with the recombinant-DNA-derived product (Poupart et al., 1987).

The results of this comparison support the view that the antiviral effect of BSF-2 reflects an epiphenomenon rather than the main biological activity of the molecule.

The amino-acid sequence of the BSF-2 molecule differs significantly from that

of IFN-β_1. Some homologies in sequence and resemblance in hydropathy maps have been brought to attention (Revel et al., 1986). However, these resemblances are not better than those between IFN-α/β and IFN-γ (De Grado et al., 1982; Dijkmans and Billiau, 1985). Therefore, on the sole basis of amino-acid sequence homologies there is no better reason to designate this molecule as an IFN-β than there would be to call it IFN-α, IFN-γ or other.

Although the in vitro translation products of 26K mRNA are precipitable with antisera prepared against semi-purified batches of IFN-β_1 (now known to be contaminated with 26K protein), the translation products are not precipitable with highly specific polyclonal antibodies against IFN-β_1 (Content et al., 1982; Zilberstein et al., 1985). Also, the natural fibroblast-derived molecule does not bind on immobilized antibodies directed against IFN-β_1, but do bind on immobilized antibodies against 26K protein itself.

In spite of this apparent failure of 26K protein to bind to antibodies against IFN-β_1 the antiviral activity of the molecule does not manifest itself when antibodies directed against IFN-β_1 are included in the system. Nonspecificity of the antibodies cannot account for this observation, since the use of monoclonal antibodies against IFN-β_1 has yielded the same result.

Currently, two hypotheses for explaining this strange phenomenon are under investigation. The first hypothesis tries to explain the antiviral effect of 26K protein by postulating that high doses of it induce production of classical IFN-β_1 which acts back on the cells rendering them resistant against virus infection. This hypothesis can explain all positive observations, including for instance the fact that 26K protein induces intracellular enzymes and cell-membrane antigens which are also induced by other interferons. It also predicts that anti-IFN-β_1-antibodies, although they may be able to neutralize the antiviral effect of high doses of 26K protein, may not be able to neutralize the B-cell stimulating effects of even low doses of the same protein. This prediction matches reality. Unfortunately, positive evidence demonstrating induction if IFN-β_1 production by 26K protein is at present not (yet) available (Zilberstein et al., 1985). Therefore, while not being totally unlikely, this hypothesis must be held in abeyance.

The second hypothesis assigns direct antiviral activity to the 26K protein by virtue of its ability to bind and trigger directly the IFN-β_1 receptor on cells. Neutralization of this antiviral activity by antibody against IFN-β_1 is explained by assuming that such antibody recognizes and blocks the site on the 26K protein which it shares with IFN-β_1 and which triggers the receptor. However, this goes counter to the observation that even the strongest available polyclonal anti-IFN-β_1 antibodies are unable to bind any 26K protein. Therefore, this hypothesis seems rather unlikely.

From these considerations it seems logical to conclude that: The 26K protein, also known as IFN-β_2, BSF-2 and HPGF, may have antiviral activity, which meets all classical but minimal criteria for the molecule to qualify as an interferon; yet, there is considerable doubt as to whether the protein directly binds and activates the receptor of IFN-β_1 (or any other known interferon receptor). In particular, the possibility that the molecule exerts its antiviral effect indirectly by a mechanism

TABLE 2

Comparison of macrophage-activating and antiviral effects of interferons α, β and γ (Literature survey).

Species	Interferon type [a]	Macrophage activity measured	Interferon dose required (units/ml)[b]	Reference
Human	α (nat)	Enhanced phagocytosis	1,000	Melby et al., 1982
Human	α/β (nat)	Tumor cell lysis	1,000–10,000	Jett et al., 1980
Mouse	α/β (nat)	Enhanced phagocytosis	10	Huang et al., 1971
Mouse	α/β (nat)	Tumor cell lysis	1,000	Männel et al., 1983
Mouse	α/β (nat)	Tumor cell lysis	10	Schultz et al., 1978
Mouse	α/β (nat)	Tumor cell lysis	1,000–10,000	Baraschi and Tagliabue, 1981
Mouse	α (nat)	Priming for tumor cell lysis	50	Pace et al., 1985
Mouse	β (nat)	Priming for tumor cell lysis	50	Pace et al., 1985
Human	γ (nat)	Class II Antigen expression	<5	Becker, 1984
Human	γ (rec)	Class II Antigen expression	<5	Basham and Merigan, 1983
Human	γ (nat, rec)	Tumor cell lysis	1–10	Le and Vilcek, 1984
Mouse	γ (rec)	Peroxide production	0.1	Nathan et al., 1983
Mouse	γ (nat)	Tumor cell lysis	0.025	Schreiber, 1985
Mouse	γ (rec)	Priming for tumor cell lysis	0.05	Pace et al., 1985
Mouse	γ (nat, rec)	Parasite killing	1	Shear, 1985

[a] nat = Natural; rec = recombinant-DNA-derived.

[b] Antiviral units.

requiring intervention of IFN-β_1 has not been excluded; on the basis of amino-acid sequence homologies and antibody-binding studies 26K protein is sufficiently different from IFN-β, as well as from IFN-α and -γ, to be excluded from either of these existing categories of substances; the dose (weight/volume) of the protein required for demonstration of antiviral effect is at least 10000-fold higher than that required for demonstration of its B-cell stimulatory effect, supporting the view that B-cell stimulation is to be considered as the main biological function of the protein.

Interferon-γ (IFN-γ)

Wheelock (1965) discovered that the supernatant fluid of leukocyte cultures, stimulated with mitogens, contain a factor(s) possessing interferon-like activity.

Physicochemically this factor appeared to stand apart from 'classical' interferon (now known as α and β), in that its activity was largely, if not entirely, lost on acidification. For a while the terms 'immune interferon' (Falcoff, 1972) and 'type II interferon' (Youngner and Salvin, 1973) came into use to designate the active principle. Eventually antisera against this interferon were produced which were able to neutralize its antiviral effect but not that of classical (type I) interferons. In reverse, antisera prepared against classical interferons were unable to neutralize the immune-induced antiviral factor. In 1980 a nomenclature committee (Stewart et al., 1980) decided to accept serological reactivity in neutralization assays as a basis to distinguish molecular types of interferons. On this basis the former designation 'type I interferon', which in the human system comprised the serologically distinct leukocyte- and fibroblast-derived interferons, was replaced by 'IFN-α' and 'IFN-β'. Application of the same and sole serological criterion led to the replacement of the term 'type II interferon' by 'IFN-γ'. This renaming implied the assumption that there was not more (or not less) difference between IFN-α and IFN-β than between either of them and IFN-γ. However, subsequent molecular and biological characterization of these molecules revealed this assumption to be wrong in that IFN-α and -β are similar in many aspects and quite different from IFN-γ. Briefly, the primary structure of IFN-α and -β shows extensive homology indicating that their genes are derived from a common ancestor; the primary sequence of IFN-γ, on the other hand, is quite different from that of either IFN-α or -β (De Grado et al., 1982; Dijkmans and Billiau 1985). The genes for all IFN-α subtypes and for IFN-β are localized on the same chromosome, both in man (chromosome 9) and in mice (chromosome 4), while the gene for IFN-γ is localized on a different chromosome (no 12 in man; no 10 in mouse) (Lovett et al., 1984; Naylor et al., 1984; Sehgal, 1982). In addition, the genes of IFN-α and -β are intronless while that for IFN-γ does contain introns (Gray and Goeddel, 1983; Gray et al., 1982; Naylor et al., 1983). The cell membrane receptor for IFN-γ is different from that for IFN-α and -β, which appear to share the same or a very similar receptor (Aguet, 1980; Anderson et al., 1982; Branca and Baglioni, 1981). Intracellular proteins induced by IFN-α and IFN-β are the same, while IFN-γ induces an additional set (Weil et

al., 1983). Important qualitative differences between IFNs-α/β and IFN-γ exist with respect to their immunomodulatory activities. IFN-α and/or -β mainly affect the activity of NK-cells, while IFN-γ mainly affects the activity of macrophages.

In view of these considerations there is now general agreement to say that IFN-α/β and IFN-γ are structurally and functionally different molecules. I will now argue that, although both types of molecules do fulfill the minimal criteria to qualify as interferons, IFN-γ may not fulfill the more critical additional criteria which have been outlined above. In particular I will try to answer two questions. Is the antiviral activity as quantified by specific activity values, of the same order of magnitude as or superior to other biological activities of the molecule?; is the antiviral effect of IFN-γ on cells a direct effect or is it partly or entirely due to induction of other cytokines?

IFN-γ is the principal T-lymphokine with macrophage-activating potential

Cells of macrophage/monocyte lineage perform important tasks in host defence against various foreign agents, in particular microorganisms, tissue transplants and cancer cells. Their activities consist mainly of (1) phagocytosis and pinocytosis, which is often mediated by receptors for the Fc fragment of immune globulins (FcR); (2) intracellular killing of ingested agents, e.g. parasites, a phenomenon which depends in part on oxidative metabolism; (3) killing of foreign cells by a non-phagocytic, contact-mediated event which may be antibody-dependent or -independent; (4) secretion of monokines (IL-1, TNF, and possibly others), which in turn exert initiating or regulatory actions on the immune system; (5) antigen presentation to lymphocytes which depends on the presence on the macrophage cell membrane of class II antigens; (6) release of proteases; (7) release of prostaglandins and leukotrienes; (8) release of superoxide components.

Resting macrophages do not exert the cited activities or do so to a limited extent only. Development of full activity, designated as activation (in a broader sense) is a multi-step process requiring two or more signals from the environment. An important signal recognized already in 1966 by Bloom and Bennet (1966) is an endogenous factor or complex of factors produced by T-lymphocytes. Various names have been given to these T-lymphokine(s) (for review see Morley et al., (1978)): MIF or migration inhibitory factor; MAF or macrophage aggregating factor; MCF or macrophage chemotactic factor; MMF or macrophage mitogenic factor are but a few. Currently, the more popular term is 'MAF' for macrophage activating factor (Weinberg et al., 1978), referring to assays using intracellular killing of parasites (Nathan et al., 1983), stimulation of oxidative metabolism (Nathan et al., 1983), enhanced expression of Class II antigens (Steeg et al., 1982), or enhanced tumor cell killing (Weinberg et al., 1978).

It has now become increasingly clear that T-cell derived MAF is indistinguishable from IFN-γ. The arguments for this are as follows: (1) ultra-pure or recombinant DNA-derived IFN-γ have been shown to possess high biological activity in various MAF assays (Basham, 1984; Nathan et al., 1983; Pace et al., 1985); (2) a linear correlation exists between titers of MAF and of IFN-γ in crude or semi-

purified T-cell supernatants of various origin (Schreiber et al., 1983; Steeg et al., 1982); (3) all of the MAF activity of crude, semipurified or pure IFN-γ preparations can be neutralized by monoclonal antibodies which also neutralize their antiviral activity (Nathan et al, 1983; Schreiber, 1985).

It is not excluded that there exist non-IFN-γ MAF's but, with current assay systems, they seem to be in a minority.

A point of crucial importance for the present discussion is whether the specific activity (biological units/weight unit) of IFN-γ is higher for its MAF-activity than for its antiviral activity. In view of the many activities which one can use to measure macrophage activation, and also in view of the various additional environmental signals which may be introduced to influence activation, it is quite understandable that assays for MAF vary widely in sensitivity and specificity. Also, at present there are no recognized international standard preparations for calibrating biological activities of mouse IFN-γ preparations. For these reasons the question raised cannot unequivocally be answered. However, an overview of the available information (see Table 2) leaves one with the impression that MAF activity of a given sample of IFN-γ is at least as high as its antiviral activity. The highest MAF-activities per unit of antiviral activity were noted when the MAF assay was made extremely sensitive by the introduction of a second signal (Schreiber, 1985).

Another question is whether the MAF activity of IFN-γ is higher than that of IFN-α and/or -β. Only one careful quantitative comparison relevant to this question is available in the literature (Pace et al., 1985). Whatever additional information is available is included in Table 2. From these studies, some of which have used MAF assay systems supposed to be highly sensitive (Boraschi and Tagliabue, 1981), it appears that for IFN-α and/or -β to exert MAF effects, they must be applied in 10- to 100-fold the minimal dose required for antiviral effects.

In conclusion, available quantitative information seems to support the view that, while all IFNs can activate macrophages, IFN-α/β do so as a side effect, while IFN-γ possesses macrophage activating potential as a principal biological function.

The indirect antiviral action of IFN-γ

The first recorded indication that IFN-γ may exert its antiviral activity through an indirect mechanism came from studies on the requirement for protein synthesis (Dianzani et al., 1980). It was shown that the establishment of the antiviral state in mouse cells following IFN-γ treatment required two rounds of protein synthesis rather than one in the case of IFN-α/β. In subsequent studies, from the same laboratory, it was shown that, under specific conditions, a large portion of the antiviral effect of mouse IFN-γ could be blocked by antibodies directed against mouse IFN-α (Hughes and Baron, 1987a,b). The specific condition seems to be that the cell monolayers are not confluent when exposed to IFN-γ. It is assumed that in confluent monolayers the antibody against IFN-α cannot reach its target as it may be trapped in the narrow spaces between cells. As a corollary the authors could show that, both in a sub-cultured mouse cell line (L929) and in fresh splenocyte cultures IFN-γ could induce production of an acid-resistant interferon-like factor, probably IFN-α and/or -β.

In discussing their results, the authors raise the obvious question why neutralization of the antiviral activity of IFN-γ by antibodies against IFN-α had not been seen in the past. Cross-neutralization assays using antisera specific for the three types of IFN are common practice in many laboratories and often form the basis for distinguishing IFN-γ from IFN-α/β in crude or semipurified preparations. Two explanations are suggested. One is that, in performing cross-neutralization assays, most authors use confluent monolayers. The second one is that they may use concentrations of anti-α or -β antibodies that are too small to reveal their neutralizing effect. A third explanation, not incompatible with the previous ones, is that various investigators did observe neutralization of IFN-γ by anti-α or -β antisera but that, out of concern for specificity, they have adjusted conditions (confluency and/or antibody dose) so as to avoid having to explain the anti-paradigmatic observation.

Given these observations, it becomes obvious that IFN-γ may exert its antiviral activity (or at least part of it) in much the same way as IL-1 i.e. by inducing other interferons.

In summary, the primary physiological role of IFN-γ seems to be its activating potential for macrophages. Secondly, it seems not excluded that the antiviral effect on cells results from induction of IFN-α and/or -β. Therefore, although the molecule fulfills the minimal criteria for being called an interferon, it may not fulfill the additional criteria which were also used to challenge the interferon status of IL-1 and 26K protein.

Epilogue

The term interferon was introduced 30 years ago to designate biologically active cellular proteins (today we would say cytokines) which mediate interference between viruses.

My overview has been focussed on recent observations which indicate that certain cytokines, e.g. IL-1, may exert interferon-like antiviral effects on cells by virtue of their ability to induce production of classical interferons, i.e. IFN-α and/or -β. Secondly, I have emphasized the fact that these cytokines have other biological activities which supersede the antiviral properties in importance and pathophysiological relevance. As typical examples for this situation I have taken IL-1, but I have argued that the same may apply to the human 26K protein, also known as IFN-β_2 (BSF-2 or HPGF), as well as to IFN-γ, which is generally considered as the main macrophage activating T-cell lymphokine.

One might therefore suggest the acceptance of two additional criteria for a cytokine to be classified as an interferon, namely (1) the cytokine must not exert its action by inducing production of a classical interferon and (2) the antiviral activity must not be superseded in quantitative (specific activity, units/mg protein) or qualitative (physiological or pathophysiological) importance by other biological activities of the molecule. However, application of the latter criterion may not always be easy, because it implies a fair deal of subjective judgement. Also, new biological actions may be revealed which open the way for challenging the interferon sta-

tus of a cytokine. Therefore, the best solution might consist in integrating the interferons in the interleukin nomenclature system. This system has indeed been designed such that it can accommodate cytokines possessing multiple biological actions. All interferons, including IFN-β_2 (26K protein) fulfill the criteria set out for a cytokine to be termed interleukins. These criteria are proposed to be as follows (Smith, 1986): (1) the substance should be produced by leukocytes (not necessarily exclusively); (2) it should have a function in host inflammatory responses to invasion by microbes and other agents; and (3) the primary amino acid sequence should be known for the human factor. It has already been suggested (Poupart et al., 1987) that the human 26K protein (IFN-β_2, BSF-2, HPGF) be called IL-6, a proposal which will probably be generally if not officially accepted when this paper appears in print. However, the arguments assembled in this overview amply justify according the interleukin status to interferon-γ as well.

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